

**PATENT COOPERATION TREATY**  
**PCT**  
**INTERNATIONAL PRELIMINARY EXAMINATION REPORT**  
(PCT Article 36 and Rule 70)

REC'D 08 JUN 2005

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Applicant's or agent's file reference Sabanci PCT 3	<b>FOR FURTHER ACTION</b>	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/TR 03/00019	International filing date (day/month/year) 20.03.2003	Priority date (day/month/year) 20.03.2003
International Patent Classification (IPC) or both national classification and IPC C12N15/62		
Applicant SABANCI UNIVERSITESI et al.		

<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 3 sheets.</p>																	
<p>3. This report contains indications relating to the following items:</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 10%; text-align: right; padding-right: 5px;">I</td> <td style="width: 90%;"><input checked="" type="checkbox"/> Basis of the opinion</td> </tr> <tr> <td style="text-align: right; padding-right: 5px;">II</td> <td><input type="checkbox"/> Priority</td> </tr> <tr> <td style="text-align: right; padding-right: 5px;">III</td> <td><input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td style="text-align: right; padding-right: 5px;">IV</td> <td><input type="checkbox"/> Lack of unity of invention</td> </tr> <tr> <td style="text-align: right; padding-right: 5px;">V</td> <td><input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td style="text-align: right; padding-right: 5px;">VI</td> <td><input type="checkbox"/> Certain documents cited</td> </tr> <tr> <td style="text-align: right; padding-right: 5px;">VII</td> <td><input type="checkbox"/> Certain defects in the international application</td> </tr> <tr> <td style="text-align: right; padding-right: 5px;">VIII</td> <td><input type="checkbox"/> Certain observations on the international application</td> </tr> </table>		I	<input checked="" type="checkbox"/> Basis of the opinion	II	<input type="checkbox"/> Priority	III	<input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	IV	<input type="checkbox"/> Lack of unity of invention	V	<input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	VI	<input type="checkbox"/> Certain documents cited	VII	<input type="checkbox"/> Certain defects in the international application	VIII	<input type="checkbox"/> Certain observations on the international application
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Date of submission of the demand 08.10.2004	Date of completion of this report 07.06.2005
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer  Seranski, P  Telephone No. +49 89 2399-7846



**INTERNATIONAL PRELIMINARY  
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**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, Pages**

1-35 as originally filed

**Claims, Numbers**

1-16 received on 13.04.2005 with letter of 13.04.2005

**Drawings, Sheets**

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.: 17-20
- the drawings, sheets:

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5.  This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

the entire international application,

claims Nos. 15-16

because:

the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (specify):

the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

no international search report has been established for the said claims Nos. 15-16

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

the written form has not been furnished or does not comply with the Standard.

the computer readable form has not been furnished or does not comply with the Standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	1-8
	No: Claims	9

Inventive step (IS)	Yes: Claims	
	No: Claims	1-14

Industrial applicability (IA)	Yes: Claims	1-14
	No: Claims	

2. Citations and explanations

**see separate sheet**

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**Re Item III**

**Non establishment of opinion with regard to novelty, inventive step and industrial applicability**

The scope of amended claims 15-16 resembles the scope of original claims 18-19 for which no search report has been established (See ISA 210). Consequently, no examination concerning novelty, inventive step and industrial applicability will be carried out.

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

The application relates to a method of immobilization, visualisation and quantification of proteins on a support material. The application aims to provide alternative to conventional His-Tag-Ni-Cellulose purification techniques. However, claimed are vectors that comprise the same elements as already known in the prior art.

Reference is made to the following documents:

- D1: WO 99 57992 A (CLONTECH LAB INC) 18 November 1999 (1999-11-18)
- D2: DE 100 13 204 A (DEUTSCHES KREBSFORSCH) 11 October 2001 (2001-10-11)
- D3: CHA HYUNG JOON ET AL: "Observations of green fluorescent protein as a fusion partner in genetically engineered Escherichia coli: Monitoring protein expression and solubility" BIOTECHNOL BIOENG;BIOTECHNOLOGY AND BIOENGINEERING 2000 JOHN WILEY & SONS INC, NEW YORK, NY, USA, vol. 67, no. 5, 2000, pages 565-574,
- D4: KEEFE ANTHONY D ET AL: "One-step purification of recombinant proteins using a nanomolar-affinity streptavidin-binding peptide, the SBP-tag." PROTEIN EXPRESSION AND PURIFICATION, vol. 23, no. 3, December 2001 (2001-12), pages 440-446, ISSN: 1046-5928

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The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-8 does not involve an inventive step in the sense of Article 33(3) PCT.

Document D1 provides vector constructs comprising Green Fluorescent Protein, a Multiple Cloning Site and an affinity peptide. The affinity peptide aims for the purification of the protein that is to be expressed by the vector construct (See.Fig.1). Said affinity peptide is specifically mentioned to be a histidine-rich polypeptide sequence.

Also document D2-D4 provide for vector constructs with a visual marker protein like GFP, a multiple cloning site, and protein tags like a His-tag or a streptavidin binding protein. All disclosed vector constructs have the property that they can produce fusion-protein that can be further immobilized, visualized and quantified.

Whether or not the interaction between the tag and the surface requires specific binding interaction is not derivable from the formulation of the claims. Independent claim 1 is formulated that it tries to define the claimed subject matter by the result to be achieved, however, the claim is not clearly limited by any concrete technical feature.

In a further aspect the specific arrangements of the vector constituents in order to achieve the desired characteristics are not defined in the claim.

The addition of the technical feature that the vector contains a frame adaptor and a spacer protein ("large distance separating protein") are standard vector design parameters very well known to the skilled person. Without any further specific technical effect linked to said features, the insertion of said elements does not contribute to an inventive step.

Consequently, claims 1-8 do not fulfil the requirements of Art. 33(3) PCT.

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 9 is not new in the sense of Article 33(2) PCT.

Document D4 discloses a construct as described supra, said construct is used in method

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of purification of a protein. The document explicitly refers to the streptavidin binding protein that can be used for detection of the recombinant protein for example in a matrix system like microtiter-plates. The streptavidin tagged protein can also be quantified as shown in the document for methods for the measurement protein-protein, protein-peptide or protein-small molecule equilibrium dissociation constants. All characterising features of claim 9 can thus be found in D4, the engineering of the construct (step a), inserting the gene of the target protein in the MCS (step b), protein expression (step c) as well as the expression and the immobilisation and washing (e-f). Steps d, g and h are optional and have therefore no limiting effect to the claimed method. Again, the type of interaction that is allegedly achieved by the claimed method if of no relevance by the assessment of novelty. Claim 9 only recited the same method steps as outlined in D4. Therefore the method of claim 9 is not new (Art.33(2) PCT).

Dependent claims 10-14 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step (Art.33(3) PCT)

AMENDED CLAIMS

- 1) Circular recombinant plasmid DNA construct encoding a) a protein tag, b) a visual marker protein, and containing c) a multiple cloning site suitable for insertion of an additional gene, characterised in that it further contains d) a frame adapter of variable length between the visual marker and protein tag genes and in that the gene sequence encoding the protein tag, the visual marker protein and the frame adaptor are specifically designed and engineered at the DNA level for respectively i) immobilisation purposes, ii) visualisation and quantification purposes at the protein level, and iii) providing a large distance separating protein and surface to enable the immobilised enzymes to display native-like characteristics.
- 10 2) Construct according to claim 1, characterised in that the protein tag is chosen from the group containing lysine (lys), histidine (his), tyrosine (tyr), phenylalanine (phe), arginine (arg), glutamic acid (glu), aspartic acid (asp), glutamate, aspartate, asparagine (asn), glycine (gly), glutamine (gln), alanine (ala), valine (val), tryptophan (trp).
- 15 3) Construct according to claim 1 or 2, characterised in that the protein tag is a histidine-tag such as a polyhistidine variant, in particular (6X) histidine.
- 20 4) Construct according to any one of the preceding claims, characterised in that the visual marker protein is chosen from the group containing fluorescent or phosphorescent proteins, wherein the fluorescent protein is chosen from the group containing Green Fluorescent Protein (GFP), Red Fluorescent Protein (RFP), Yellow Fluorescent Protein (YFP) and Blue Fluorescent Protein (BFP) as well as their variants and/or mutants.
- 5) Construct according to any one of the preceding claims, characterised in that the multiple cloning site contains restriction enzyme recognition sites chosen from the group containing SacI, SalI, HidIII, EagI, NotI.
- 25 6) Construct according to any one of the preceding claims, characterised in that it expresses a fusion protein, wherein the tag is suitable to interact directly with appropriate surface pendant groups of a support material.
- 7) Construct according to claim 6, characterised in that the direct interaction with the support material is covalent or non-covalent.
- 30 8) Construct according to claim 7, characterised in that the direct interaction is non-covalent and yet freely accessible and leach-free like covalent one.
- 9) Method for preparing and immobilising a protein on a support material, comprising:
  - a) Engineering at the DNA level, in series a protein tag suitable to interact directly with appropriate surface pendant groups of a support material, a fluorescent marker

protein for visualisation and quantification purposes at the protein level and a multiple cloning site suitable for insertion of a target protein to be immobilised,

b) Inserting the corresponding gene of the target protein to be immobilised into the multiple cloning site;

5 c) Initiating protein expression.

d) Optionally pre-treating the support material;

e) Incubating the protein and support material together, wherein the protein is immobilised to the support via specific tag-surface interactions;

f) Washing away the non-specific biomolecules;

10 g) Optionally quantifying the fluorescence of the visual marker protein;

h) Optionally desorbing the target protein.

characterised in that the support material is chosen from the group containing polymers, biopolymers, glass and composites containing silicone dioxides, metals and metal oxides, as well as any combination thereof on the microscopic, mesoscopic or 15 macroscopic length scale.

10) Method according to claim 9, characterised in that the support material is chosen from the group containing polymers, silicon dioxides, aluminum oxides, titanium oxides, magnesium oxides, borates, metals and other metal oxides.

11) Method according to any one of claims 9 and 11, characterised in that the polymers are chosen from the group containing polyolefins such as polystyrene, polyacrylates, polymethacrylates, polybutylene, polyvinylalcohol and related derivatives, polyvinylchlorides, polyisoprene, polypropylene, polyphenols, polyarnides, polyesters polysulfones, polyethersulfones, polyethersulfides, polyimines, polyethyleneglycols, polypropyleneglycols, polyimides, polycarbonates, polyurethanes.

25 12) Method according to claim 11, characterised in that the polymer surface is chemically treated to bear various functional groups chosen between carboxyl groups, hydroxyl groups, amino groups, amide groups, ester groups, imide groups, imine groups, mercapto groups, nitro groups, sulfonate groups, phosphate groups, phosphonate groups, cyano groups, sulfone groups, aldehyde groups, epoxide groups, urethane groups, ketone groups, phenolic groups, aromatic groups, alkyl, alkenyl, alkynyl, acyl and aryl groups, silanol groups, silicon oxide groups, siloxane groups, metal hydroxide groups, metal oxide groups, and elemental metals.

30 13) Method according to any one of claims 9 to 12, characterised in that the support material is carboxylated polystyrene.

14) Method according to claim 9, characterised in that quantifying in step g) of the fluorescence of the visual marker protein is used in applications selected from analysis, diagnosis (like in enzyme-based diagnostic kits), incubation, storage, sensing, arraying and orienting, catalysis, stabilisation, binding, signal transduction, chemical transformation, implant passivation and surface biocompatibilization, surface activation, purification, detoxification and scavenging.

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15) A two-component system obtained by any one of claims 9 to 14.

16) A two-component system according to claim 15 described by and an activated support material and a protein encoded by a recombinant plasmid DNA construct according to any one of claims 1 to 8

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